GE Healthcare

Instructions 28-9634-12 AB **Packed bed chromatography**

Capto™ DeVirS

Capto DeVirS is part of GE Healthcare's Custom Designed Media program and can be used for capture and intermediate purification of viruses by packed bed chromatography.

Capto DeVirS offers the following benefits:

- Excellent productivity
- Good chemical stability
- Affinity-like behavior to various viruses

Capto

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1 Custom Designed Media (CDM)

GE Healthcare Process media cover all purification steps from capture to polishing. Capto DeVirS is a process medium developed and supported for production scale chromatography. Secure ordering and delivery routines assure a reliable supply of media. This medium is included in the GE Healthcare media program for CDM.

2 Properties of Capto DeVirS

Capto DeVirS is an affinity medium with the ligand dextran sulfate which is known to have an affinity-like behavior to different types of virus.

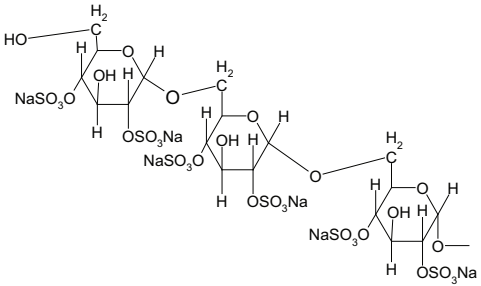
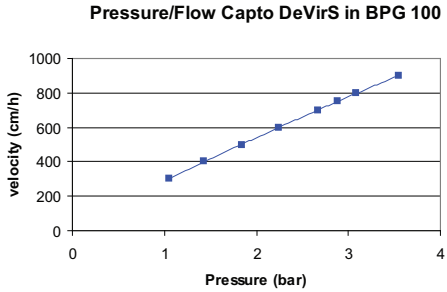


Fig 1. Capto DeVirS consists of highly cross-linked agarose base matrix coupled to dextran sulfate as ligand.

Capto DeVirS is designed for large scale purification of viruses in capture and/or intermediate purification. The ability to run at high flow velocities with low backpressure can shorten process cycle times and increase productivity.

The medium is based on a high-flow agarose matrix. Typical flow velocities at large scale (1 m column diameter and 20 cm bed height) are 600 cm/h, with a backpressure below 3 bar (Fig 2). The highly cross-linked agarose base matrix gives the medium high

chemical and physical stability. Characteristics such as capacity, elution behavior and pressure/flow properties are unaffected by the solutions commonly used in process chromatography when purifying virus (Table 1).



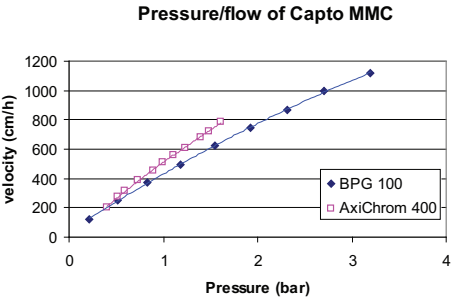


Fig 2. The first figure shows Capto DeVirS being packed in BPG 100 to 20 cm bed height. Pressure flow data is generated in water at 20°C. The second figure shows Capto MMC packed under the same conditions but also packed in AxiChrom 400, pressure/flow run in water at 20°C. Capto DeVirS and Capto MMC have the same base matrix and Capto DeVirS is expected to show a similar performance when scaled up to Axichrom 400.

Table 1. Characteristics of Capto DeVirS

Matrix	Highly cross-linked agarose
Functional group	Dextran sulfate
Ligand density	70-130 µmol S/ml drained gel
Particle size [1]	75 µm (d_{50v})
pH stability[2, 3]	
• Short term (2 h)	6 to 14
• Long term (1 wk)	7 to 13
Working temperature [4]	4°C to 30°C
Chemical stability	All commonly used aqueous buffers, 0.1 M sodium hydroxide. [5]
Avoid	Low pH

[1] d_{50v} is the median particle size of the cumulative volume distribution.

[2] Short-term pH: pH interval that the medium can be subjected to, for cleaning or sanitization-in-place (accumulated 50 hours (100 cycles) at room temperature) 10% decrease in dynamic capacity, less than 20% loss of sulfur content.

[3] Long-term pH: pH interval that the medium can be operated at without significant change in sulfur content.

[4] Capto DeVirS can be used under cold-room conditions. This is not tested but there should not be any stability issues due to low temperature.

[5] No significant decrease in sulfur content after 1 week storage in 0.1 M NaOH at 40°C.

3 Method design and optimization

The aim of designing and optimizing an affinity method for the separation of biomolecules is to identify conditions that promote binding of the highest amount of target with the highest possible product recovery as well as impurity clearance.

Design of Experiment (DoE) is an effective tool for investigation of the effect of several parameters on virus binding, recovery and clearance of HCP and DNA in order to establish the optimal purification protocol.

It may be necessary to screen for optimal loading conductivity and pH with respect to binding capacity and impurity clearance. Capto DeVirS does not allow binding at high conductivity. When working with influenza virus the conductivity is recommended to be below 5 mS/cm and the pH is recommended to be between 6.8 and 7.8. It may be optimal to use a different pH at loading than at elution.

For elution it may also be necessary to screen for optimal conditions, especially pH. HTPD plates could be a suitable format for screening the different parameters.

4 Suggested purification protocol

- Concentrate the clarified feed with ultrafiltration and perform a buffer exchange to the start buffer, for example 20 mM sodium phosphate pH 6.8, by diafiltration.
- Equilibrate the column with start buffer.
- Apply the sample to the column.
- Wash out unbound sample using start buffer.
- Elute the virus with elution buffer, for example 20 mM sodium phosphate comprising 1.5 M NaCl pH 7.4.

5 Optimization of throughput

Balancing product recovery against throughput is the major consideration when optimizing a method. The dynamic binding capacity for the target virus should be determined. Since the dynamic binding capacity is a function of the flow velocity applied during sample application, the binding capacity must be defined over a range of different residence times (flow velocities) to identify the optimum level of throughput.

6 Scaling up

After optimizing the method at laboratory scale, the process can be scaled up.

1. Select the bed volume according to required binding capacity.
2. Select a column diameter to obtain a bed height of 10 - 45 cm. To utilize the full potential of Capto DeVirS, we recommend bed heights of 20 cm or higher at large scale.
3. Scale-up is typically done by keeping bed height and flow velocity constant, while increasing bed diameter and volumetric flow rate. However, since optimization is preferentially done with small column volumes, in order to save sample and buffer, some parameters like the dynamic binding capacity may be optimized using shorter bed heights than those being used in the final scale. As long as the residence time is constant, the binding capacity for the target molecule remains the same.

Other factors, like clearance of critical impurities, may change when column bed height is changed and should be validated using the final bed height. The residence time is approximated as the bed height (cm) divided by the flow velocity (cm/h) applied during sample loading.

7 Packing process-scale columns

See application note *Methods for packing Capto MMC in production scale columns* for process-scale column packing instructions. See Chapter 11 "Ordering information" for article number.

8 Packing lab-scale columns

The following instructions are for packing lab-scale columns: Tricorn 10/100 with 10 cm bed height and XK 16/40 with 20 cm bed height.

For more details about packing Tricorn columns, see *Tricorn Empty High Performance Columns* or for a practical instruction in good packing techniques, the CD ROM *Column Packing - The Movie*.

See Chapter 11 "Ordering information" for article numbers.

Packing Tricorn 10/100 and XK 16/40 columns

Materials

Chemicals

- Capto DeVirS
- Distilled water
- 99.5% ethanol
- Acetone
- Sodium chloride
- Potassium sulfate

Equipment

- Spatula or plastic pipette
- Thin capillary
- Glass filter funnel
- Vacuum suction equipment
- Suction bottle

- Pressure monitor
- Measuring cylinder or beaker
- Lab stand

Columns

- Tricorn 10/100 GL columns
- Tricorn packing kit with 10/100 GL, 10/150 GL, 10/300 GL columns as packing reservoirs
- XK 16/40 column with fixed and adjustable bottom unit and adapter head

See Chapter 11 "Ordering information" for article numbers.

Preparing the gel

1 Equilibrate all material to room temperature.

2 Capto DeVirS media is supplied in 20% ethanol. Suspend the gel by shaking and pour into a graduated glass filter or a graduated laboratory beaker.

Note: *The gel slurry must be homogeneous.*

Washing the gel

1 Mount the glass filter funnel onto the filtering flask.

2 Pour the gel slurry into the funnel and wash according to the following instructions - be sure to suction the gel dry after each step:

1 Distilled water: 5 times with one gel volume

2 Slurry/packing solution: 5 times with one gel volume

Packing in Tricorn 10/100

Main features of the method used:

- Slurry/packing solution: 0.25 M KSO_4
- Slurry concentration: 50%
- Settling flow: 25 ml/min (1900 cm/h) 5 min
- Compression flow: 25 ml/min = 2 min
- Modified adapter head mounting technique
- Adapter adjustment: 2/3 turn

Packing procedure

To pack the column, use 0.25 M KSO4 in purified water and proceed as follows:

1. Assemble the column according to the column instruction that comes with the column.
2. Put a stop plug into the bottom of the column tube and pour the chromatography medium into the top of the packing tube, filling both column tube and packing tube. Make sure there are no air bubbles in the medium.
3. Attach an extra bottom unit or an adapter unit to the top of the packing tube. Place a beaker beneath the column tube and connect a pump to the top of the packing unit. Remove the stop plug from the bottom of the column tube.
4. Pack the medium at 25 ml/min (1910 cm/h) for 5 minutes.
5. When the medium is packed, switch off the pump, attach the stop plug into the bottom of the column tube, disconnect the pump and remove the packing tube and packing connector. If necessary, remove excess medium by resuspending the top of the packed bed and removing the medium with a Pasteur pipette or spatula.
6. Fill up the column with the same fluid as used for packing the column.
7. Place a prewet filter on top of the fluid in the column and gently push it into the column tube with the filter tool.
8. Prepare the adapter unit by screwing the guiding ring inside the adapter unit out to its outer rim and then turn it back 1.5 turns.
9. Wet the O-ring on the adapter unit by dipping it in water, buffer or 20% ethanol.
10. Screw the adapter unit onto the column tube, ensuring the inner part of the guiding ring fits into the slot on the column tube threads. Ensure that there are no air bubbles.
11. Screw the adapter down to approximately 1 mm above the surface.
12. Connect the pump to the adapter unit. Remove the stop plug from the bottom of the column.

13 Repack the medium at 25 ml/min (1910 cm/h) for > 2 min and slowly screw the adapter down until the filter reaches the bed surface.

14 Switch off the pump, attach the stop plug into the bottom of the column tube and disconnect the pump. Screw the adapter unit down for a further 2/3 turns into the bed.

15 Press the adapter lock down into the locked position.

16 Screw a stop plug into the adapter unit.
The column is now ready for use or storage.

Plate test Tricorn 10/100

Table 2. Plate test parameters for Tricorn 10/100

Equilibration	CV of 20% ethanol at a flow rate of 2.0 m/min
Sample volume	100 µl
Sample concentration	2% v/v acetone in 20% ethanol
Test flow velocity	30 cm/h (0.4 ml/min)
Detection	UV at 280 nm
System	ÄKTA Explorer (UNICORN 5.x)

See Chapter 9 "Evaluation of column packing".

Packing XK in 16/40

Main features of the method used:

- Slurry/Packing solution: 0.25 M KSO_4
- Slurry concentration: 50%
- Packing flow: 40 ml/min (900 cm/h) 5 min
- After packing flow: 40 ml/min ≥ 5min
- Compression: 10-15% (of bed height)

Packing procedure

To pack the column, use 0.25 M KSO4 in purified water and proceed as follows:

1 Wet the bottom filter with 20% ethanol with the aid of a syringe mounted on the outlet tubing. After the filter is wetted, mount a stop screw on the outlet.

2 Mount the bottom piece onto the column tube.

3 Wet the adapter filter with 20% ethanol with the aid of a syringe mounted on the inlet tubing. After the filter is wetted, mount a stop screw on the inlet.

4 Mount the column vertically on a stand.

5 Fill the glass tube with medium slurry up to the upper edge of the glass tube.

6 Mount the adapter to the upper part of the glass tube with no air trapped below the filter.

7 Tighten the sealing ring and connect the column inlet to the system outlet.

8 Open the bottom outlet of the column.

9 Pack the column at 900 cm/h (40 ml/min, XK 16) for 5 min.

Note: *Do not exceed the operating pressure limit for the columns (5 bar for XK 16).*

Note: *If a packing reservoir is used: Stop the pump and mount a stop screw on the outlet. Disconnect the packing reservoir and repeat steps 7-9.*

10 Pack the column at 900 cm/h (40 ml/min, XK 16) for > 5 min.

11 Mark the bed height on the glass tube with a pen.

12 Stop the pump and mount a stop screw on the outlet.

13 Adjust the adapter quickly down to approximately 1 cm above the medium surface with the O-ring loose.

14 Tighten the O-ring and adjust the adapter down to the mark and further 15 mm into the medium bed. Screw a stop plug into the adapter unit.
The column is now ready for use or storage.

Plate test XK 16/40

Table 3. Plate test parameters for XK 16/40

Equilibration	3 CV of 20% ethanol at a flow rate of 4.0 m/min
Sample volume	500 µl
Sample concentration	2% v/v acetone in 20% ethanol
Test flow velocity	30 cm/h (1 ml/min)
Detection	UV at 280 nm
System	ÄKTA Explorer (UNICORN 5.x)

See Chapter 9 "Evaluation of column packing".

9 Evaluation of column packing

Test column efficiency to check the quality of the packing. Tests should be made directly after packing and at regular intervals during the working life of the column, as well as when separation performance is seen to deteriorate. The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). These values are easily determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 0.8 M NaCl in water with 0.4 M NaCl in water as eluent.

Note: *The calculated plate number will vary according to the test conditions and it should only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc. will influence the results.*

For optimal results, the sample volume should be a maximum of 2.5% of the column volume and the flow velocity between 15 and 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column use.

Method for measuring HETP and A_s

Calculate HETP and A_s from the UV curve (or conductivity curve) as follows:

$$HETP = \frac{L}{N}$$

$$N = 5.54 \times \left(\frac{V_R}{W_h}\right)^2$$

where:

L = bed height (cm)

N = number of theoretical plates

V_R = volume eluted from the start of sample application to the peak maximum

W_h = peak width measured as the width of the recorded peak at half of the peak height

V_R and W_h are in the same units

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height, h, is calculated as follows:

$$h = \frac{HETP}{d}$$

where:

d is the diameter of the bead

As a guideline, a value of <3 is very good.

The peak should be symmetrical, and the asymmetry factor as close to 1 as possible (values between 0.8–1.5 are usually acceptable).

A change in the shape of the peak is usually the first indication of bed deterioration due to use.

Peak asymmetry factor calculation:

$$A_s = \frac{b}{a}$$

where:

a = ascending part of the peak width at 10% of peak height

b = descending part of the peak width at 10% of peak height

Figure 3 shows a UV trace for acetone in a typical test chromatogram in which the HETP and A_s values are calculated.

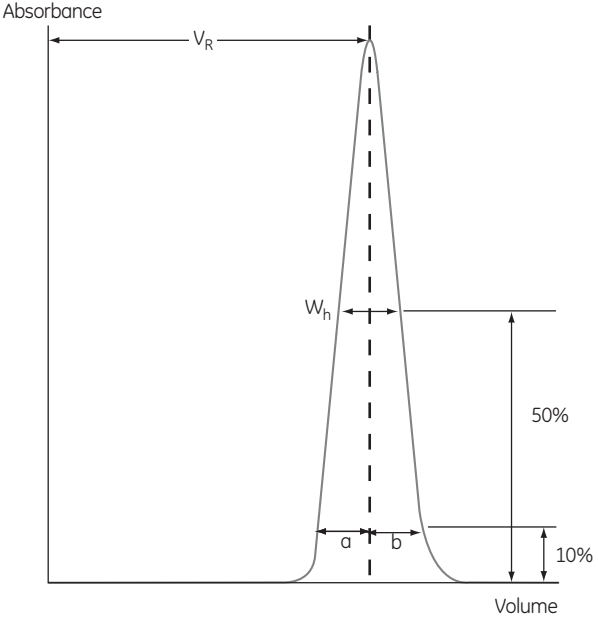


Fig 3. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

10 Maintenance

For the best performance from Capto DeVirS over a long working life, follow the procedures described below.

Equilibration

After packing, and before a chromatographic run, it is recommended to perform a blank run including cleaning-in-place (CIP). Equilibrate the column with start buffer by washing with at least 5 bed volumes, or until the column effluent shows stable conductivity and pH values.

Cleaning-in-place

Cleaning-in-place (CIP) is a procedure that removes contaminants such as lipids, endotoxins and precipitated or denatured proteins that remain in the packed column after regeneration. These types of contamination occur frequently when working with crude feedstock. Regular CIP prevents the build-up of contaminants in the medium bed and helps to maintain the capacity, flow properties and general performance of Capto DeVirS.

A specific CIP protocol should be designed for each process according to the type of contaminants present. The frequency of CIP depends on the nature and the condition of the feedstock, but for capture steps CIP is recommended after each cycle. The recommended CIP is 1 M NaOH for at least 30 min, in reversed flow.

Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5-1.0 M NaOH with a contact time of 1 hour is recommended.

Autoclave

It is possible to autoclave Capto DeVirS without any significant loss of function when using sodium acetate or sodium phosphate buffer.

Storage

Store unused media in the container at a temperature of 2°C to 8°C. Ensure that the screw top is fully tightened. Packed columns should be equilibrated in 20% ethanol to prevent microbial growth and ensure neutral pH and stored at a temperature of 2°C to 8°C. After storage, equilibrate with at least 5 bed volumes of starting buffer before use.

11 Ordering information

Capto DeVirS

Product	Pack size	Article No.
Capto DeVirS *	25 ml	17-5466-01
Capto DeVirS *	100 ml	17-5466-02
Capto DeVirS *	1 liter	17-5466-03
Capto DeVirS *	5 liter	17-5466-04

* This product is part of GE Healthcare's Custom Designed Media program.

* All bulk media products are supplied in suspension in 20% ethanol. For additional information, including data file, please contact your local GE Healthcare representative.

Related products

Product	Quantity	Article No.
Tricorn 10/100 column	1	28-4064-15
XK 16/40 column	1	18-8774-01

Accessories

Product	Quantity	Article No.
Tricorn 10 coarse filter kit	1	11-0012-54
Tricorn packing connector 10/100	1	18-1153-25
Tricorn 10/150 glass tube	1	18-1153-16
Tricorn 10/300 glass tube	1	18-1153-18

Related literature

Product	Quantity	Article No.
Application note: Methods for packing Capto MMC in production scale columns	1	28-9259-33
Column Packing - The Movie	1	18-1165-53
Data file: Capto DeVirS	1	28-9616-49
Tricorn Empty High Performance Columns instruction	1	28-4094-88

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First published Sep. 2009.

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28-9634-12 AB 04/2010